AF ZFW

BARNES & THORNBURG LLP

11 South Meridian Street
Indianapolis, Indiana
46204
(317) 236-1313
(317) 231-7433 Fax

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Application No.:	10/074,169
Invention:	AUTOMATED ANALYSIS OF REAL-TIME NUCLEIC ACID AMPLIFICATION
Inventor:	Carl T. Wittwer
Filed:	February 12, 2002
Attorney Docket:	7475-70049
Examiner:	Jeffrey N. Fredman

Certificate Under 37 CFR 1.8(a)

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on June 21, 2006

Rebecca Ball
(Signature)

Rebecca L. Ball
(Printed Name)

CORRECTED APPEAL BRIEF

Mail Stop Appeal Brief
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

This is an appeal to the Board of Patent Appeals and Interferences from the Primary Examiner's May 23, 2005 Final Rejection of claims 1 and 3-10. Appellant believes that no fees are required for this filing. If any fees are required, the Commissioner is hereby authorized to charge any additional fees or credit any overpayment to Appellant's undersigned counsel's deposit account 10-0435 with reference to our matter 7475-70049. A duplicate copy of this authorization is enclosed for this purpose.

REAL PARTY IN INTEREST

The real party in interest is the University of Utah Research Foundation, by virtue of assignments recorded on April 16, 2002 and May 31, 2002 in the records of the Patent and Trademark Office on patent record reel 012830, beginning at frame 0368, and on patent record reel 012935, beginning at frame 0896, respectively.

RELATED APPEALS AND INTERFERENCES

There are no related appeals or interferences.

STATUS OF CLAIMS

In response to the office action mailed on November 28, 2003, claims 11-13 have been canceled. In response to the office action mailed on June 15, 2004, claim 2 was canceled. In response to the office action mailed on June 15, 2004, claims 1 and 3 were amended. Claims 1 and 3-10, all of the claims remaining in this application, are rejected. Claims 1 and 3-10 are subject to a Final Rejection, from which this appeal was taken. An Appendix containing a list of the claims on appeal is attached to this appeal brief.

STATUS OF AMENDMENTS

No amendments were filed subsequent to the Final Rejection from which this appeal is taken.

SUMMARY OF CLAIMED SUBJECT MATTER

The invention may best be understood by referring to the following summary of independent claims 1 and 10, annotated with exemplary references to the specification.

Claim 1 relates to a method for determining the presence of a nucleic acid in a sample (page 3, lines 1-2). The method comprises the steps of providing a fluorescent entity capable of indicating the presence of the nucleic acid and capable of providing a signal related to the quantity of the nucleic acid (page 3, lines 2-4), amplifying the nucleic acid through a plurality of amplification cycles in the presence of the fluorescent entity (page 3, lines 4-5), measuring fluorescence intensity of the fluorescent entity at each of the plurality of amplification cycles to produce a fluorescent value for each cycle related to the quantity of the nucleic acid present at each cycle (page 3, lines 6-8), generating a plot wherein the fluorescent values are recorded for each amplification cycle (page 3, lines 8-9), performing a confidence band analysis on the plot to generate a positive or negative call (page 3, lines 9-10), and if the call is positive, confirming the positive call by a melting temperature analysis (page 3, lines 10-11), wherein the confidence band analysis is performed by calculating slopes of segments of the plot using a plurality of the fluorescent values (page 16, lines 21-22), using the segment slopes of the plot to establish a baseline fluorescence region by generating a slope value for each of a plurality of the amplification cycles (page 16, lines 23-24), and establishing the baseline fluorescence region comprising an interval of cycles that includes the amplification cycle with the slope value having an absolute value closest to zero (page 16, lines 25-27), and making the positive or negative call based on whether the fluorescence value during a selected amplification cycle is outside the baseline fluorescence region (page 16, lines 28-29).

Claim 10 relates to an automated method for determining the presence of a nucleic acid (page 3, lines 16-17). The method comprises the steps of placing a sample into a

container containing a fluorescent entity capable of indicating the presence of the nucleic acid and capable of providing a signal related to the quantity of the nucleic acid (page 3, lines 17-19), placing the container into a device for amplifying the nucleic acid through a plurality of amplification cycles in the presence of the fluorescent entity (page 3, lines 19-21), measuring fluorescence intensity of the fluorescent entity at each of the plurality of amplification cycles to produce a fluorescent value for each cycle related to the quantity of the nucleic acid present at each cycle (page 3, lines 21-24), generating a plot wherein the fluorescent values are recorded for each amplification cycle (page 3, lines 24-25), calculating slopes of segments of the plot using a plurality of the fluorescent values (page 3, lines 25-26), using the segment slopes of the plot to establish a baseline fluorescence region by generating a slope value for each of a plurality of the amplification cycles (page 3, lines 26-28), and establishing the baseline fluorescence region comprising an interval of cycles that includes the amplification cycle with the slope value having an absolute value closest to zero (page 3, lines 28-30), outputting a positive result if the fluorescence value of a selected amplification cycle is outside the baseline fluorescence region (page 3, lines 30-31), and confirming the positive result by melting temperature analysis (page 3, lines 31-32).

GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

The grounds of rejection to be reviewed by the Board are 1.) whether claims 1 and 3-10 are unpatentable for obviousness-type double patenting over claims 1-24 of U. S. Patent 6,387,621 (hereinafter the '621 patent) in view of Herrmann et al., and 2.) whether claims 1 and 3-10 would have been obvious under 35 U.S.C. § 103 based upon Wittwer EP 1059523 A2, (the European counterpart of the '621 patent) in view of Herrmann et al.

ARGUMENT

Rejection of Claims 1 and 3-10 for Obviousness-Type Double Patenting

The Examiner has rejected claims 1 and 3-10 for obviousness-type double patenting over claims 1-24 of the '621 patent in view of Herrmann et al. Thus, the Examiner is suggesting that the subject matter of claims 1 and 3-10 is an obvious variant of the invention claimed in the '621 patent in view of the disclosure of Herrmann et al. The Appellant respectfully traverses the Examiner's rejection of claims 1 and 3-10 for obviousness-type double patenting. Claims 1 and 3-10 are not obvious over the '621 patent claims in view of Herrmann et al.

The claims of the present application specify at least the steps of generating a plot wherein the fluorescence values are recorded for each amplification cycle, performing a confidence band analysis on the plot to generate a positive or negative call, and if the call is positive, confirming the positive call by a melting temperature analysis.

Generally, the '621 patent claims are directed to performing a polymerase chain reaction (PCR) in which a baseline fluorescence region is established by confidence band analysis, and ascertaining whether the fluorescence value during a selected amplification cycle is outside the baseline fluorescence region. As conceded by the Examiner, the '621 patent claims do not specify "confirming the results by using a melting temperature analysis." See page 3, lines 15-16 of the May 23, 2005 Office Action. The Examiner cites Herrmann et al. to provide the teaching of using a melting temperature analysis because the '621 patent claims lack this teaching.

There is no motivation to combine the '621 patent claims with Herrmann et al. As conceded by the Examiner, the '621 patent claims do not specify "confirming the results by using a melting temperature analysis." See page 3, lines 15-16 of the May 23, 2005 Office Action. With respect to Herrmann et al., the Examiner indicates that Herrmann et al. teaches

"performing a PCR reaction followed by confirming the target using a melting temperature analysis." See page 3, lines 17-18 of the May 23, 2005 Office Action. Thus, neither the '621 patent claims nor Herrmann et al. expressly states that the two steps of 1.) generating a positive call by confidence band analysis and 2.) using melting temperature analysis as a second confirmation step should be combined.

The Examiner cites *Ruiz v. A.B. Chance Company* (Fed. Cir. 2004) in which the court stated that an "an examiner may find a motivation to combine prior art references in the nature of the problem to be solved." The Examiner relies on *Ruiz* as a basis for the argument that there is motivation to combine the '621 patent claims and Herrmann et al. because, according to the Examiner, there are express statements in the '621 patent and in Herrmann et al. that suggest that Herrmann et al. solves the problem recognized in the '621 patent.

Thus, the Examiner states that the "nature of the recognized problem in U.S. Patent No. 6,387,621 lends itself to solution by combination with the Hermann reference." See page 11, lines 16-18 of the May 23, 2005 Office Action. To support his motivation to combine arguments, the statements that the Examiner points to in Herrmann et al. are that "[t]he ability to multiplex PCR analysis by color and T_m has many uses in addition to multiplex genotyping. For example, internal amplification controls are often needed for infectious disease and translocation testing to verify that amplifiable DNA or cDNA is present even if the target amplification is negative. Another common need is for multiplexing a competitor as an internal standard for PCR quantification (see page 428, column 1)." See page 4, lines 14-19 of the May 23, 2005 Office Action.

To support his motivation to combine arguments, the statements that the Examiner points to in the '621 patent are that "accurately discriminating between positive and negative samples is not easy in practice (see column 6, lines 15-16)," and that

"[a]utomatic identification of the background is surprisingly difficult. (see column 6, lines 48-49)." See page 5, lines 3-6 of the office action. The Examiner argues that Herrmann et al. provides an additional solution for the problem recognized in the '621 patent that accurately discriminating between positive and negative PCR samples is difficult.

According to the Examiner, the statements discussed above provide motivation to a skilled artisan to combine Herrmann et al. with the '621 patent claims because the '621 patent indicates that accurately discriminating between positive and negative samples is difficult, and Herrmann et al. provides a solution to this problem by "providing a means to accurately discriminate between positive and negative PCR samples." See page 5, lines 7-8 of the May 23, 2005 Office Action.

First, the statements in the '621 patent that "accurately discriminating between positive and negative samples is not easy in practice," and that "[a]utomatic identification of the background is surprisingly difficult" do not, *when taken in the context of the '621 patent*, motivate a skilled artisan to combine the invention of the '621 patent claims with Herrmann et al. or with any other reference that describes confirmatory methods for identifying PCR amplification products. The '621 patent has provided a solution to the problem that "accurately discriminating between positive and negative samples is not easy in practice." The solution to this problem that is provided by the '621 patent is confidence band analysis to generate a positive or negative call. There is no suggestion in the '621 patent that any additional verification or confirmatory method, in addition to confidence band analysis, is needed to discriminate between positive and negative PCR samples.

The statements in the '621 patent that the Examiner points to that "accurately discriminating between positive and negative samples is not easy in practice," and that "[a]utomatic identification of the background is surprisingly difficult" are found in column 6, lines 15-17 and column 6, lines 48-49 of the '621 patent. These statements are made in a

section of the '621 patent that discusses problems with prior art methods that had previously been used to attempt to discriminate between positive and negative PCR samples. These statements are in a section of the '621 patent that leads into the specific description in the '621 patent of the steps that are performed to carry out confidence band analysis according to the method described in the '621 patent. This specific description of the steps in the confidence band analysis method described in the '621 patent begins at column 6, line 55 and continues through column 8, line 67. The last statements in this section of the '621 patent are that "[i]f the test point fluorescence is outside of the confidence interval, the sample is positive. If it is within the interval, the sample is negative. FIGS. 7 and 8 are samples which are positive, while FIGS. 9-11 are negative samples." See column 8, lines 64 to 67 of the '621 patent.

There is no suggestion in the '621 patent that the confidence band analysis method described in the '621 patent has not solved the problem addressed in the '621 patent or that any additional verification or confirmatory method is needed. In fact, the last statements, prior to the '621 patent claims, describing the specific steps of the confidence band analysis method that is the subject of the '621 patent are that "[i]f the test point fluorescence is outside of the confidence interval, *the sample is positive.* If it is within the interval, *the sample is negative.*" See column 8, lines 64 to 66 of the '621 patent. (Emphasis added). These statements provide no indication that the results of the confidence band analysis method described in the '621 patent could be inaccurate in any way or that a second verification or confirmation is needed. Once a result has been ***confirmed***, the result does not need to be confirmed again. To the contrary, the statements that if a fluorescence value is outside of the confidence band interval, *the sample is positive*, and if the value is within the confidence band interval, *the sample is negative*, indicate that the results of the confidence band analysis method described in the '621 patent are conclusive.

The Examiner has taken the statements in the '621 patent that "accurately discriminating between positive and negative samples is not easy in practice," and that "[a]utomatic identification of the background is surprisingly difficult" out of the context in which those statements are made in the '621 patent. These statements are made in a section of the '621 patent that discusses the problem that is addressed in the '621 patent, but that is resolved by the confidence band analysis method described in the '621 patent. The confidence band analysis method described in the '621 patent is the solution to the problem that "accurately discriminating between positive and negative samples is not easy in practice."

There is no suggestion in the '621 patent that the confidence band analysis method described in the '621 patent has not solved the problem pointed to by the statements that "accurately discriminating between positive and negative samples is not easy in practice," and that "[a]utomatic identification of the background is surprisingly difficult." There is simply no statement in the '621 patent that definitively suggests that the confidence band analysis method described in the '621 patent has not solved the problem addressed in the '621 patent and the Examiner has not pointed to such a statement. Indeed, Appellant respectfully submits that the only suggestion that an additional confirmatory method is needed to verify the results obtained by confidence band analysis is the Examiner's hindsight through the lens of Appellant's own disclosure in the present application, and such hindsight analysis is not allowed. The '621 patent is devoid of any suggestion that an additional confirmatory method is needed to verify the results obtained by confidence band analysis. It is Appellant's own disclosure in the present application that first suggests that an additional confirmatory method (*i.e.*, melting temperature analysis) may be needed to verify the results obtained by confidence band analysis.

In his September 17, 2005 Advisory Action, the Examiner contends that the

argument that the '621 patent is satisfied with its method, taken to its logical conclusion, "would gut section 103" because "virtually every patent is satisfied with its method and, thus, no improvements would ever be obvious." To the contrary, virtually every patent states that the prior art is unsatisfactory. If an Examiner could use statements in a parent patent about unsatisfactory aspects of the prior art, in combination with a reference that discloses the new element of Applicant's claims, regardless of the context in which the new element is disclosed, no improvements would ever be patentable. The issue is whether there is any suggestion in the '621 patent that the invention claimed in the '621 patent is unsatisfactory. There is no such suggestion in the '621 patent. The Examiner is taking statements made in the '621 patent out of context and is attempting to turn statements in the '621 patent that the prior art is unsatisfactory into statements that invention described and claimed in the '621 patent is unsatisfactory.

Furthermore, even if the '621 patent suggested that confidence band analysis does not solve the problem described in the '621 patent, and, again Appellant contends that there is simply no such suggestion in the '621 patent, the nature of the problem pointed to in the '621 patent would not lead a skilled artisan to look to Herrmann et al. for a solution. The '621 patent describes a method for accurately discriminating between ***positive and negative samples*** based on an analysis of the background fluorescence during a PCR reaction. Herrmann et al. has nothing to do with analyzing background fluorescence to discriminate between positive and negative PCR samples, but, rather involves ***differentiating between multiple DNA's in a sample***. Accurate discrimination between positive and negative samples in the context of the '621 patent is accomplished ***without analysis or comparison to different signals resulting from different nucleic acids in the sample***. In contrast, melting temperature analysis in the context of Herrmann et al. is used to determine the presence of a nucleic acid in a sample by analyzing and comparing different signals resulting from different

nucleic acids in the sample.

In fact, Herrmann et al. is limited to the use of melting curve analysis in the context of discriminating between multiple DNA's (*i.e.*, multiple signals) in a PCR sample. Herrmann et al. solves a completely different problem than is pointed to in the '621 patent. In this regard, the statements cited by the Examiner in Herrmann et al. that the Examiner argues provide express motivation to combine Herrmann et al. with the '621 patent by providing a solution to the problem identified in the '621 patent are all directed to using melting temperature analysis to ***discriminate between multiple DNA's in a PCR sample***. The statements in Herrmann et al. pointed to by the Examiner are:

> The ability to multiplex PCR analysis by color and T_m has many uses in addition to multiplex genotyping. For example, internal amplification controls often are needed for infectious disease and translocation testing to verify that amplifiable DNA or cDNA is present even if the target amplification is negative. Another common need is for multiplexing a competitor as an internal standard for PCR quantification.

See page 428, last paragraph of Herrmann et al.

The statements cited by the Examiner in Herrmann et al. are directed to discrimination between multiple DNA's 1.) in the context of multiplex genotyping, 2.) in the context of discrimination between an internal amplification control and the target DNA, and 3.) in the context of discrimination between a competitor as an internal standard and the target DNA. These statements in Herrmann et al. are unambiguously limited to the use of melting curve analysis in the context of ***multiplex experiments***. In other words, Herrmann et al. is unambiguously limited to the use of melting temperature analysis to discriminate between multiple DNA's in a PCR sample. The methods described in Herrmann et al. have nothing to do with analyzing background fluorescence to discriminate between positive and negative PCR samples. Thus, the nature of the problem pointed to in the '621 patent would

not lead a skilled artisan to look to Herrmann et al. for a solution, and there is no motivation to combine these references.

The Examiner cites additional statements in the '621 patent and in Herrmann et al. to support his argument that the nature of the problem pointed to in the '621 patent would lead a skilled artisan to look to Herrmann et al. for a solution, and further that statements in Herrmann et al. would lead to the combination of Herrmann et al. with the '621 patent. The Examiner states on page 10, lines 3-6 of the office action that Herrmann et al. teaches that a single nucleic acid could be analyzed using the method described in Herrmann et al. because Herrmann et al. states that "Probes of a single color are usually used for genotyping" (see page 425, line 6 of Herrmann et al.).

Again, the Examiner has taken a statement out of the context in which it is made in the reference. The statement that immediately follows the statement referred to by the Examiner on page 425, line 6 of Herrmann et al. is that "[f]our alleles at two different loci have been genotyped by multiplexing probe T_ms of a single color." Thus, in the proper context Herrmann et al. states that:

> Probes of a single color are usually used for genotyping. Four alleles at two different loci have been genotyped by multiplexing probe T_ms of a single color.

These statements together indicate that probes of a single color have been used to identify four different alleles by ***multiplex genotyping*** which again refers to discriminating between ***multiple DNA's*** in a sample as discussed above. Thus, the statement that "[p]robes of a single color are usually used for genotyping" does not at all teach that a single nucleic acid could be analyzed by the method described in Herrmann et al. as the Examiner contends.

Furthermore, the Examiner cites an additional statement in the '621 patent to support an argument that the nature of the problem pointed to in the '621 patent would lead a

skilled artisan to look to Herrmann et al. for a solution. The Examiner indicates on page 10, lines 6-13 of the office action that claim 11 of the '621 patent teaches allele comparisons. However, claim 11 specifies a method where the nucleic acid is further analyzed to determine the presence of "a particular allele." Claim 11 provides no indication that multiple alleles are compared and the '621 patent specification provides no indication that the method of claim 9 or claim 11 is used for multiplex genotyping. Thus, claim 11 of the '621 patent teaches single allele identification and does not teach multiple allele comparisons as contended by the Examiner.

As discussed above, the Examiner has taken the statements in the '621 patent that "accurately discriminating between positive and negative samples is not easy in practice," and that "[a]utomatic identification of the background is surprisingly difficult" out of the context in which those statements are made in the '621 patent to support his argument that the '621 patent suggests that confirmatory methods in addition to confidence band analysis are needed to accurately discriminate between positive and negative PCR samples. Again, there is simply no statement in the '621 patent that suggests that the confidence band analysis method that is the subject of the '621 patent has not solved the problem described in the '621 patent and the Examiner has not pointed to such a statement. The only suggestion that a confirmatory method in addition to confidence band analysis may be needed to accurately discriminate between positive and negative PCR samples is through the Examiner's hindsight based on review of Appellant's present application, and such hindsight analysis is improper.

Moreover, the nature of the problem pointed to in the '621 patent would not lead a skilled artisan to look to Herrmann et al. for a solution because the methods described in Herrmann et al. have nothing to do with discriminating between positive and negative PCR samples based on analysis of background fluorescence, and this is the problem described in

the '621 patent. Accordingly, there is no motivation to combine the '621 patent and Herrmann et al. either based on express statements in the '621 patent or in Herrmann et al. or based on the nature of the problem to be solved, and the Appellant's claimed method is not obvious over the '621 patent claims in view of Herrmann et al.

Rejection of Claims 3, 5, 6, and 9 for Obviousness-Type Double Patenting

In addition to the arguments discussed above, claims 3, 5, 6, and 9 specify establishing the baseline fluorescent region "without the use of an internal standard" (claim 3), obtaining the melting profile "by monitoring fluorescence between extension and denaturation during one of the amplification cycles" (claim 5), obtaining the melting profile "by monitoring fluorescence between annealing and denaturation during one of the amplification cycles" (claim 6), and obtaining the melting profile "by monitoring fluorescence at temperature increments of greater than 0.1°C" (claim 9). Appellant submits that claims 3, 5, 6, and 9 are patentable for the same reasons as noted above. Also, Appellant wishes to point out that neither the '621 patent claims nor Herrmann et al. provides any teaching or suggestion of the limitation of establishing the baseline fluorescent region "without the use of an internal standard," as recited in claim 3, obtaining the melting profile "by monitoring fluorescence between extension and denaturation during one of the amplification cycles," as recited in claim 5, obtaining the melting profile "by monitoring fluorescence between annealing and denaturation during one of the amplification cycles," as recited in claim 6, or obtaining the melting profile "by monitoring fluorescence at temperature increments of greater than 0.1°C," as recited in claim 9.

Rejection of Claims 1 and 3-10 Under 35 U.S.C. § 103(a)

The Examiner also rejected claims 1 and 3-10 under 35 U.S.C. § 103(a) over Wittwer in view of Hermann et al. Wittwer is the European counterpart of the '621 patent. The Examiner made the same substantive arguments for rejecting claims 1 and 3-10 under 35 U.S.C. § 103(a) based on Wittwer in view of Herrmann et al. as the Examiner made for rejecting claims 1 and 3-10 for obviousness-type double patenting. Accordingly, all of Appellant's arguments made above with respect to the obviousness-type double patenting rejection apply with equal force to this rejection except that the text of Wittwer is applicable rather than the claims of the '621 patent.

CONCLUSION

Accordingly, Appellant submits that the Examiner's rejections of claims 1 and 3-10 for obviousness-type double patenting and for obviousness under 35 U.S.C. §103(a) are clearly erroneous. Appellant urges that the Board reverse the Examiner's rejections. Such action is respectfully requested.

Respectfully submitted,

Rebecca L. Ball
Attorney Reg. No. 46,535
Attorney for Appellant

Indianapolis, Indiana
(317)231-7511

CLAIMS APPENDIX

1. A method for determining the presence of a nucleic acid in a sample comprising the steps of

providing a fluorescent entity capable of indicating the presence of the nucleic acid and capable of providing a signal related to the quantity of the nucleic acid,

amplifying the nucleic acid through a plurality of amplification cycles in the presence of the fluorescent entity,

measuring fluorescence intensity of the fluorescent entity at each of the plurality of amplification cycles to produce a fluorescent value for each cycle related to the quantity of the nucleic acid present at each cycle,

generating a plot wherein the fluorescent values are recorded for each amplification cycle,

performing a confidence band analysis on the plot to generate a positive or negative call, and

if the call is positive, confirming the positive call by a melting temperature analysis,

wherein the confidence band analysis is performed by

calculating slopes of segments of the plot using a plurality of the fluorescent values,

using the segment slopes of the plot to establish a baseline fluorescence region by generating a slope value for each of a plurality of the amplification cycles, and establishing the baseline fluorescence region comprising an interval of cycles that includes the amplification cycle with the slope value having an absolute value closest to zero, and

making the positive or negative call based on whether the fluorescence value during a selected amplification cycle is outside the baseline fluorescence region.

3. The method of claim 1 wherein the baseline fluorescent region is established without the use of an internal standard.

4. The method of claim 1 wherein the melting temperature analysis is performed by

obtaining a melting profile,

determining the minimum or maximum of the first derivative to generate a Tm value, and

comparing the Tm value with the known Tm of the target analyte.

5. The method of claim 4 wherein the melting profile is obtained by monitoring fluorescence between extension and denaturation during one of the amplification cycles.

6. The method of claim 4 wherein the melting profile is obtained by monitoring fluorescence between annealing and denaturation during one of the amplification cycles.

7. The method of claim 4 wherein the melting profile is obtained by monitoring fluorescence in a separate melting process subsequent to amplification.

8. The method of claim 4 wherein the melting profile is obtained by monitoring fluorescence at 0.1°C temperature increments.

9. The method of claim 4 wherein the melting profile is obtained by monitoring fluorescence at temperature increments of greater than 0.1°C.

10. An automated method for determining the presence of a nucleic acid comprising the steps of

placing a sample into a container containing a fluorescent entity capable of indicating the presence of the nucleic acid and capable of providing a signal related to the quantity of the nucleic acid,

placing the container into a device for amplifying the nucleic acid through a plurality of amplification cycles in the presence of the fluorescent entity,

measuring fluorescence intensity of the fluorescent entity at each of the plurality of amplification cycles to produce a fluorescent value for each cycle related to the quantity of the nucleic acid present at each cycle,

generating a plot wherein the fluorescent values are recorded for each amplification cycle,

calculating slopes of segments of the plot using a plurality of the fluorescent values,

using the segment slopes of the plot to establish a baseline fluorescence region by generating a slope value for each of a plurality of the amplification cycles, and establishing the baseline fluorescence region comprising an interval of cycles that includes the amplification cycle with the slope value having an absolute value closest to zero,

outputting a positive result if the fluorescence value of a selected amplification cycle is outside the baseline fluorescence region, and

confirming the positive result by melting temperature analysis.

EVIDENCE APPENDIX

No evidence has been submitted in this case pursuant to 37 C. F. R. §§ 1.130-1.132.

RELATED PROCEEDINGS APPENDIX

There are no copies of decisions rendered by a court or the Board in any proceedings identified pursuant to 37 C. F. R. § 41.37(c)(1)(ii).

CLAIMS AS THEY STOOD AT THE TIME THE APPEAL BRIEF WAS FILED:

1. (Previously presented) A method for determining the presence of a nucleic acid in a sample comprising the steps of

providing a fluorescent entity capable of indicating the presence of the nucleic acid and capable of providing a signal related to the quantity of the nucleic acid,

amplifying the nucleic acid through a plurality of amplification cycles in the presence of the fluorescent entity,

measuring fluorescence intensity of the fluorescent entity at each of the plurality of amplification cycles to produce a fluorescent value for each cycle related to the quantity of the nucleic acid present at each cycle,

generating a plot wherein the fluorescent values are recorded for each amplification cycle,

performing a confidence band analysis on the plot to generate a positive or negative call, and

if the call is positive, confirming the positive call by a melting temperature analysis,

wherein the confidence band analysis is performed by

calculating slopes of segments of the plot using a plurality of the fluorescent values,

using the segment slopes of the plot to establish a baseline fluorescence region by generating a slope value for each of a plurality of the amplification cycles, and establishing the baseline fluorescence region comprising an interval of cycles that includes the amplification cycle with the slope value having an absolute value closest to zero, and

making the positive or negative call based on whether the fluorescence value during a selected amplification cycle is outside the baseline fluorescence region.

2. (Canceled)

3. (Previously presented) The method of claim 1 wherein the baseline fluorescent region is established without the use of an internal standard.

4. (Original) The method of claim 1 wherein the melting temperature analysis is performed by

obtaining a melting profile,

determining the minimum or maximum of the first derivative to generate a Tm value, and

comparing the Tm value with the known Tm of the target analyte.

5. (Original) The method of claim 4 wherein the melting profile is obtained by monitoring fluorescence between extension and denaturation during one of the amplification cycles.

6. (Original) The method of claim 4 wherein the melting profile is obtained by monitoring fluorescence between annealing and denaturation during one of the amplification cycles.

7. (Original) The method of claim 4 wherein the melting profile is obtained by monitoring fluorescence in a separate melting process subsequent to amplification.

8. (Original) The method of claim 4 wherein the melting profile is obtained by monitoring fluorescence at 0.1°C temperature increments.

9. (Original) The method of claim 4 wherein the melting profile is obtained by monitoring fluorescence at temperature increments of greater than 0.1°C.

10. (Original) An automated method for determining the presence of a nucleic acid comprising the steps of

placing a sample into a container containing a fluorescent entity capable of indicating the presence of the nucleic acid and capable of providing a signal related to the quantity of the nucleic acid,

placing the container into a device for amplifying the nucleic acid through a plurality of amplification cycles in the presence of the fluorescent entity,

measuring fluorescence intensity of the fluorescent entity at each of the plurality of amplification cycles to produce a fluorescent value for each cycle related to the quantity of the nucleic acid present at each cycle,

generating a plot wherein the fluorescent values are recorded for each amplification cycle,

calculating slopes of segments of the plot using a plurality of the fluorescent values,

using the segment slopes of the plot to establish a baseline fluorescence region by generating a slope value for each of a plurality of the amplification cycles, and establishing the baseline fluorescence region comprising an interval of cycles that includes the amplification cycle with the slope value having an absolute value closest to zero,

outputting a positive result if the fluorescence value of a selected amplification cycle is outside the baseline fluorescence region, and

confirming the positive result by melting temperature analysis.

11-13. (Cancelled)